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Application of surface plasmon resonance imaging technique for the detection of single spherical biological submicrometer particles

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ABSTRACT

Recent proof-of-principle studies demonstrated the suitability of the surface plasmon resonance imaging (SPRi) technique for the detection of individual submicrometer and nanoparticles in solutions. In the current study, we used the SPRi technique for visualization of the binding of round-shaped viruses (inactivated influenza A virus) and virus-like particles (human immunodeficiency virus (HIV)-based virus-like particles) to the functionalized sensor surface. We show the applicability of the SPRi technique for the detection of individual virus-like particles in buffers without serum as well as in buffers containing different concentrations of serum. Furthermore, we prove the specificity of visualized binding events using two different pseudotypes of HIV virus-like particles. We also demonstrate the applicability of the SPRi technique for the determination of relative particle concentrations in solutions. Moreover, we suggest a technical approach, which allows enhancing the magnitude of binding signals. Our studies indicate that the SPRi technique represents an efficient research tool for quantification and characterization of biological submicrometer objects such as viruses or virus-like particles, for example.

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Surface plasmon resonance (SPR) is a label-free and highly sensitive optical method of analysis, which is widely used to study interactions between different types of biomolecules (peptides, proteins, nucleic acids). In the classical SPR approach, interactions between immobilized molecules and floating target–analyte lead to the formation of layers of biomolecules onto the sensor surface. Formation of such layers affects the refractive index near the sensor surface. In turn, changes of the refractive index serve as a basis for the real-time measurements of the binding efficiency of biomolecules. Thus, SPR-based sensors have been broadly used in such areas as determination of affinity constants (in development and characterization of antibodies, for example) or certain concentration measurements of target biomolecules. For a long time, SPR-based sensors remained useless for the detection of binding of single nanoscale particles to the functionalized sensor surface. This restriction is mainly associated with a problem of the lateral resolution described for SPR-based sensors [1–3]. It was affirmed that the length of plasmonic propagation is crucial for SPR resolution and, thus, limits the minimal size of detected objects to a micrometer scale. Therefore, biological submicrometer and nanoscale objects such as viruses or virus-like particles (VLPs) and their characteristics remained practically out of the scope of SPR-based research work.

Only recent groundbreaking studies performed in independent working groups helped in realizing the potential of SPR-based sensors in studies of biological submicrometer and nanoparticles [4–7]. Zybin and colleagues used SPR-based "microscopy" (also called "SPR nanoscopy") to detect the binding of individual polystyrene particles of different sizes (minimal used size was 40 nm diameter) to the functionalized sensor surface [6]. The same working group detected the binding of single human immunodeficiency virus (HIV)-VLPs (around 100–140 nm diameter) to the antibody-coated sensor surface [6]. Wang and colleagues demonstrated the ability

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of SPR-based sensors to detect single influenza A viral particles in solutions [5]. Moreover, the authors demonstrated that virus–surface interactions depend on the type of sensor functionalizing and also applied the SPR technique for the determination of the mass and size of investigated influenza A particles [5]. Further, Halpern and colleagues used the SPR-based sensor for the real-time imaging of single DNA-functionalized gold nanoparticle hybridization adsorption events [7].

A prime advantage of the SPR-based imaging technique is its ability to detect signals attributed to viral particles by themselves, not to the structural proteins or genetic material of the particles. Thus, with this method instant information about the existence of target biological submicrometer particles in analyzed samples can be obtained. Besides this characteristic, the SPRi method has another crucial advantage: in comparison with culture assays or other direct methods: the SPR-based assay can be performed within minutes and only in the case of very low concentrations of viruses requires hours for viral detection [6]. However, direct methods often require long turnaround time (days) and intensive labor procedures. In comparison with indirect methods such as ELISA or nucleic acid amplification tests (PCR, RT-PCR for example), the SPR-based technique also has certain advantages. As indirect methods, ELISA and PCR-based assays do not provide sufficient information regarding the amount of intact virus particles and thus the question of quantitative interpretation of received results remains open. Moreover, irrespective of anticipated concentrations these methods require relatively long turnaround time (hours) and in certain cases (RT-PCR) labor-intensive procedures and highly qualified personal. Thus, SPR-based sensor appears to be especially useful in research areas, when precise quantifications of intact biological particles are crucial [8]. In addition, it is noteworthy that despite the fact that low concentration samples were not used in the current research work, the detection limit of the SPR-based sensor may be significantly better compared to the current study as discussed in the previous work [6].

Certain key questions regarding bioanalytical characteristics of SPR-based sensors still remained open and we wanted to address them. Therefore, we investigated in the present work whether biological objects of different shape may be visualized using an SPR-based sensor. We studied the selectivity of the SPR-based sensor using samples with two different pseudotypes of HIV-VLPs. Moreover, we checked whether the SPR-based detection of biological submicrometer particles remains possible in complex buffers containing different serum concentrations. We also estimated the responsiveness of the SPR-based sensor to the changes of HIV-VLP's concentration in samples and evaluated the suitability of the SPR-based sensor for particle concentration measurements. Finally, we suggest an approach, which may improve the detectability of biological submicrometer objects by SPR-based sensors.

Materials and methods

Materials

Phosphate-buffered saline (PBS) buffer was prepared by mixing of sterile water (Aqua B. Braun, Melsungen, Germany) with 10X DPBS (Gibco, Life Technologies). FBS was purchased from Invitrogen. Nüscofloc, a liquid containing around 10% of aluminum hydroxide chloride was purchased from Dr. Nüsgen Chemie (Dr. Nüsken Chemie, Germany). After filtration this solution was used for coverage of a gold sensor surface in the experiments with polystyrene particles. Thus, here and further, this filtrated solution is named "aluminum hydroxide chloride." Cell culture medium for HEK293T cells was based on Dulbecco's modified Eagle medium (Gibco, Life Technologies) supplied with 10% FBS (Invitrogen), penicillin (Life Technologies), and streptomycin (Life Technologies). Sucrose was received from AppliChem (AppliChem, Germany) and was used during VLP purification to make a 20% solution in 1X DPBS. Sodium chloride was received from Sigma Aldrich, (Sigma Aldrich, Germany). Tubes and pipettes were purchased from Eppendorf (Eppendorf, Germany). Filter tips were purchased from StarLab (StarLab GmbH, Germany). Some other used reagents and materials were purchased from Thorlabs, PHASIS, RatioLab, Behr Labor-Technik, and VWR International.

Implementation of surface plasmon resonance experimental setup

The experimental setup is described in detail in our previous publications [4,6]. In brief, a Kretschmann scheme [9] for plasmon excitation is used. In contrast to the classical SPR, in our SPR-based sensor the individual bound particles are visualized and detected instead of measuring the resonance angle shift caused by formation of the layer of particles onto the sensor area.

Glass slides were covered with titanium and further covered with a gold layer (PHASIS, Switzerland). The thickness of the metal layer was about 50 nm consisting of 5 nm Ti and approximately 45 nm gold. Deposition was performed using a magnetron-sputtering technique. These glass slides are named as "gold sensors." During experiments, a gold sensor was attached to the glass prism with refractive index $n = 1.725$ using an immersion liquid (Cargille Laboratories Inc., USA) with the same refractive index. Further, the gold layer was illuminated through the glass prism by a collimated beam of the superluminescent diode QSDM-680-9 (QPhotonix, USA) or by a diode laser HL6750MG (Thorlabs GmbH, Germany). In both cases the exciting wavelength of the incidence beam was $\lambda \approx 675$ nm. An incidence angle was chosen on the left (smaller angle) slope of the resonance minimum. A 50 mm Minolta Rokkor MD photo-objective with an aperture of 1/1.7 was used for the imaging of the gold surface onto a video camera. In the current studies, a 5-megapixel GC2450 Prosilica camera (Allied Vision) with Sony ICX625 CCD image sensor (3.45×3.45 μm pixel size) is used. Magnification factor was chosen ≈6, so that one pixel corresponded to approximately 0.6 μm on the sensor surface.

The flow-cell system was made and used as described previously [6]. Analytes were pumped through the U-shaped flow cell as a suspension either in distilled water containing 0.3% sodium chloride (for 100 nm polystyrene particles sulfate latex 8% w/v from Invitrogen, USA) or in PBS (for HIV virus-like particles, inactivated influenza A virus (IAV), or tobacco-mosaic virus (TMV)). For pumping silicon hosepipes (Spectec, Germany; ID of the product: 0.508 mm) and a 4-channel peristaltic pump Rabbit Peristaltic Pump (Rainin Instruments, France) with a velocity of 0.3 ml/min were used. Before and after each experiment, the flow-cell system was washed with PBS or with water depending on the type of the planned or performed experiment. Image recording speed used during the experiments with 100 nm polystyrene particles was 40–41 frames per second.

The Streampix 3.0 (NorPix, Canada, www.norpix.com) software was used to record the images. Image processing was performed either manually using ImageJ software (http://rsb.info.nih.gov/ij) or using the software created by the authors [10,11]. The detailed processing of images using the ImageJ software is described in [4,6]. A signal appears as a bright spot on the gray background image since a bound particle disturbs the evanescent field of the surface plasmon–polariton wave and thus affects the local reflectivity. It is necessary to wait until the binding rate becomes stable and afterward the signal counting can be started. Counting rate is the number of signal spots for which a step of at least 40 arbitrary units (a.u.) in the intensity value is detected during observation

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